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Brief Report

Longitudinal changes of serum cytokines in patients with chronic low back pain and Modic changes

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SUMMARY

Objectives: To explore serum cytokine levels over time in patients with chronic low back pain (cLBP) and Modic changes (MCs), difference in change between treatment groups in the Antibiotics in Modic Changes (AIM) study and associations between change in cytokines and low back pain.
Methods: Serum concentrations of 39 cytokines were measured at baseline and 1 year from 73 participants in the AIM study; 30 randomized to placebo, 43 to Amoxicillin. Low back pain intensity was measured by numeric rating scale. Change in cytokine levels over time were assessed by paired *t*-tests. Difference in change in cytokine levels between treatment groups and associations between changes in LBP and cytokine levels were assessed by linear regression models. Networks of cytokine changes in each treatment groups were explored by Pearson's correlations.
Results: Five cytokines changed from baseline to 1 year, (mean change, log transformed values with CI) C-X-C motif chemokine ligand (CXCL) 10 (IP-10) (0.11 (0.01–0.20)), CXCL13 (0.61 (0.00–0.12)), C-C motif chemokine ligand (CCL)26 (0.05 (0.01–0.1)), granulocyte macrophage-colony stimulating factor (GM-CSF) (−0.12 (−0.23 to 0.00)) and CXCL11 (0.12 (0.03–0.22)). Treatment group only influenced change in CCL21 (β 0.07 (0.01–0.12)), and IL-6 (β −0.17 (−0.30 to −0.03)). Change in CXCL13 (β 2.43 (0.49–4.38)), CCL27 (β 3.07 (0.46–5.69)), IL-8 (β 1.83 (0.08–3.58)) and CCL19 (β 3.10 (0.86–5.43)) were associated with change in LBP. The correlation networks of cytokine changes demonstrate small differences between treatment groups.
Conclusions: Cytokine levels are relatively stable over time in our sample, with little difference between treatment groups. Some cytokines may be associated with LBP intensity. The differences between the correlation networks suggest that long-term Amoxicillin-treatment may have longstanding effects to be further explored.

Abbreviations: cLBP, chronic low back pain; MCs, Modic changes; AIM-study, Antibiotics in Modic Changes study.

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Introduction

Low back pain (LBP) is the leading cause of disability worldwide and affects all age groups causing activity limitation and work absence with a subsequent enormous economic burden[1]. Even though the majority of acute LBP episodes have good prognosis, a significant number of patients experience recurrent episodes and approximately 10% develop chronic complaints classified as non-specific chronic LBP (cLBP)[2]. Finding relevant cLBP subgroups could help understanding disease mechanisms, predict prognosis, choice of treatment and tailoring future therapies.

Modic changes (MCs) are common magnetic resonance imaging (MRI) findings in patients with LBP[3], and proposed as a relevant cLBP phenotype. MCs are classified into type 1 (oedema type), 2 (fatty type) and 3 (sclerotic type) based on T1-and T2 weighted MRI[3]. The aetiology is unknown but there is evidence for inflammatory mediators being implicated in the development of MCs[4], and studies report up-regulation of cytokines in the disc and serum in patients with cLBP and MCs[5,6].

In a recent study of participants from the AIM-study (a randomized, placebo-controlled trial investigating the effect of Amoxicillin in patients with cLBP and MCs type 1 or type 2) we identified increased serum concentrations of inflammatory cytokines in patients with cLBP and MCs compared to healthy controls[5]. The aim of this longitudinal study with repeated blood sampling was to explore: 1) if there were changes in cytokine levels over time, 2) whether such changes differed between placebo and long-term treatment with Amoxicillin and 3) if there were associations between change in cytokines and change in LBP.

Materials and methods

Study population

This study uses data from a subsample of patients in the AIM-study[7]. The AIM-study randomized 180 outpatients to either 3 months oral treatment of 750 mg Amoxicillin three times daily or placebo. Full details of the inclusion criteria have been published elsewhere[5,7,8]. Seventy-three patients had available prospective blood samples for the cytokine sub-study (30 in the placebo group, 43 in the Amoxicillin group).

Ethical considerations

The AIM-study was approved by the Regional Committees for Medical Research Ethics in South East Norway (ref nr 2014/158), registered at ClinicalTrials.gov (NCT02323412), and monitored by the Clinical Trial Unit, Oslo University Hospital. All participants gave written informed consent.

Blood sampling

Blood was collected from AIM-participants at screening and at 1 year follow-up in BD Vacutainer®tubes with no additives that were stored in room temperature for 45 min before centrifugation at 2000 *g* for 10 min at room temperature. Serum was immediately aliquoted and stored at −80°C prior to cytokine analyses.

Cytokine analyses

We determined the concentrations of 40 cytokines by duplicate serum analysis (high-sensitivity detection) with a 40-plex Pro Human Chemokine multi-bead assay (Cat. no.: 171 AK99MR2, Bio-Rad, Norway). Data was recorded with a Luminex IS 100 instrument (Bio-Rad, Hercules, CA, USA) and protein concentrations were determined using recombinant standard curves.

Clinical assessments

We used patient reported outcome measures filled out at baseline and at 1 year follow-up to assess LBP, disability, general health and psychological factors[7].

Statistics

All analyses were done using SPSS version 27, STATA version 16 and R.

Being an exploratory study, we did not adjust for multiple testing, and an alpha of 0.05 was applied to minimize the risk of type 2 errors.

The cytokine levels were natural log-transformed as most cytokines were not normally distributed, and also to reduce the impact of outliers. Within-comparison of cytokine levels between baseline and 1 year was performed by a paired *t*-test between time points. A linear regression model was applied to assess the difference in change in cytokine levels between treatment groups, using change in individual cytokine level as dependent variable and treatment group as independent variable adjusting for baseline value of the relevant cytokine, MC type and previous low back surgery.

Furthermore, we applied a principal component analysis (PCA) on the total sample on differences in serum levels of all cytokines from baseline to 1 year.

To explore underlying patterns of cytokine networks in the placebo and Amoxicillin group we did Pearson's correlation to assess interrelatedness between serum changes of all cytokines in each treatment group. Network plots were generated from adjacency matrices in the placebo and Amoxicillin groups.

To assess the relationship between change in pain and change in cytokine levels, we did regression analyses with change in LBP [numerical rating scale (NRS)] from baseline to 1 year as the dependent variable, and change in individual cytokine levels as independent variables adjusting for possible confounders as baseline cytokine values, age, body mass index (BMI), gender, comorbidity and psychological factors (Fear Avoidance Beliefs Questionnaire and Hopkins Symptom Checklist).

Results

Characterization of the samples

There were no baseline-differences in characteristics between the Amoxicillin and placebo group (Table I). CXCL5 was below the limit of quantification for more than half of the samples and excluded from further analyses.

Comparison of cytokine levels from baseline to 1 year

There was a change in serum cytokine levels over time for four cytokines, CXCL13, CCL26, GM-CSF and CXCL11. CXCL13, CCL26 and CXCL11 were decreased after 1 year, whereas GM-CSF increased (Suppl Table 1 + Suppl Fig. 1).

Between group differences

Results from the regression model of how treatment group influence individual cytokine change from baseline to 1 year are shown in Fig. 1. There was a change in CCL21 (β 0.07 (0.01–0.12), $P = 0.02$) and IL-6 (β −0.17 (−0.30 to −0.03), $P = 0.02$), i.e., patients

	Placebo $n = 30$	Antibiotics $n = 43$
Demographic characteristics of patients		
Sex, female	19 (63.6%)	25 (58.1%)
Age, years	45.0 ± 9.2	44.1 ± 8.3
BMI*	26.2 ± 4.7	25.6 ± 3.2
Smoking, yes	8 (26.7%)	15 (34.9%)
Characteristics of LBP		
RMDQ baseline*	12.7 ± 4.1	12.6 ± 4.9
RMDQ 1 year†	11.1 ± 6.6	10.0 ± 5.9
NRS baseline	6.2 ± 1.5	6.4 ± 1.2
NRS 1 year†	5.2 ± 2.3	5.0 ± 2.1
Duration of LBP, years	2.9 (3.9)	2.8 (5.5)
CRP baseline	1.3 (1.7)	1.2 (2.4)
General health and psychological factors, baseline		
Hopkins symptom checklist	1.5 (0.7)	1.5 (0.6)
EQ-5D	0.5 ± 0.2	0.6 ± 0.2
FABQfa*	18.3 ± 13.8	17.0 ± 11.5
FABQw*	20.0 ± 13.8	17.0 ± 11.5
Comorbidities		
Diabetes	0	0
Arthritis, osteoarthritis	1 (3.3%)	3 (7.0%)
Asthma	2 (6.7%)	1 (2.3%)
Myocardial infarction	1 (3.3%)	1 (2.3%)
Reflux, peptic ulcer, gastritis oesophagitis	2 (6.7%)	3 (7.0%)
Depression	4 (13.3%)	1 (2.3%)
Use of analgesics		
Paracetamol	2 (6.7%)	6 (14.0%)
Paracetamol + codeine	2 (6.7%)	2 (6.7%)
Opioids	3 (10%)	4 (9.3%)
NSAIDs	8 (26.7%)	11 (25.6%)
Modic type		
Modic type 1	13 (43,3%)	25 (58,1%)
Modic type 2	17 (56,7%)	18 (41,9%)

RMDQ = Roland Morris Disability Questionnaire, EQ-5D = EuroQol-5D, FABQ = fear-avoidance beliefs Questionnaire (FABQpa questions related to physical activity, FABQw questions related to work), NSAIDS = non-steroidal anti-inflammatory drugs, BMI = body mass index, CRP = c reactive protein, NRS = numerical rating scale.
Continuous variables are given as mean ± standard deviation (SD) or median with interquartile range if not normally distributed. Categorical variables are given with absolute and relative frequencies.
* Placebo $n = 29$, antibiotics $n = 43$.
† Placebo $n = 27$, antibiotics $n = 43$.

Table I

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Characteristics of patients with serum cytokine profiles at baseline and 1 year

in the Amoxicillin group had a positive change in CCL21 compared to placebo, whereas a negative change in IL-6, meaning a lower serum concentration of IL-6 at 1 year compared to the placebo group.

Principal component analysis

Overall, there was no clustering of patients in either group ($n = 73$ samples and $n = 39$ cytokines), although some patients separate in the two first PCs (Suppl Fig. 2).

Correlations of serum cytokine changes in placebo and treatment group

Since cytokines interact in tightly regulated networks to mediate and regulate various cellular processes, we performed Pearson's correlation analyses of changes in serum cytokine levels in the treatment groups separately. The significant results from these analyses are plotted as correlation heatmaps in Suppl Fig. 3 and reveal different directionality of correlation patterns of changes. The patterns differ visually, as there are two larger clusters of correlated cytokine changes appearing in the Amoxicillin group, whereas in the placebo group there is one large cluster, and two smaller clusters. Also, some cytokine changes are negatively correlated in the Amoxicillin group.

To further visualize the networks, we performed network analysis and plotted network graphs of cytokines displaying strong correlation of changes (>0.7 Pearson correlation coefficient) (Suppl Fig. 4). This analysis shows that the change in levels of CXCL9, CXCL10 (IP-10) and CXCL11 form a network. These cytokines form a network with tumor necrosis factor(TNF) and CCL19 in the placebo group, whereas in the Amoxicillin group they interplay with IL-10 and CCL8. For the placebo group, the changes in the CXCL9-CXCL10 (IP-10)-CXCL11-TNF network is not correlated to changes to other cytokines, whereas for the Amoxicillin group, the network contains several other cytokines. There are small networks with only two cytokines in both the placebo (CCL22, CXCL12) and treatment (CCL11, CCL2) group, of unknown significance.

Associations between change in low back pain and change in serum cytokine levels

Changes in serum concentrations of four cytokines, CCL27, IL-6, IL-8 and CCL19, were associated with change in LBP (NRS) over

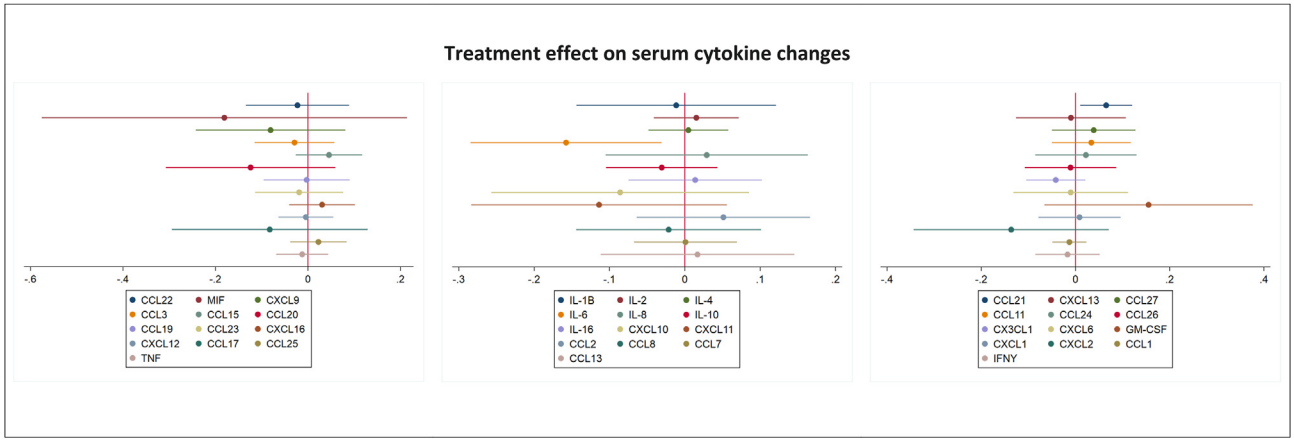

Fig. 1 Osteoarthritis and Cartilage

Coefficient plots of treatment effect with confidence intervals for each individual cytokine. The figure gives an overview of the association between treatment group and change in cytokine levels showing coefficient plots with confidence intervals for each individual cytokine. Positive values means change (1 year minus baseline) in serum cytokine levels are positive in the Amoxicillin compared to the placebo group, and negative values means change (1 year minus baseline) in s-cytokine levels are negative in the Amoxicillin compared to the placebo group. The analyses are adjusted for Modic type, previous surgery and baseline values of the relevant cytokine.

1 year in an unadjusted model. In the adjusted model IL-6 was no longer associated with change in LBP intensity, whereas change in CXCL13 became statistically significantly associated (Suppl Tabl 2).

Discussion

A recent study found increased levels of several cytokines in patients with MC compared with healthy controls, indicating that inflammation plays a role in MC biology[5]. In the present study we found that serum cytokine concentrations in patients with cLBP and MCs were relatively stable over time. The between group changes in serum cytokine concentrations from baseline to 1 year was evident for only two cytokines; IL-6 and CCL21, and the biological and clinical relevance is uncertain. The PCA did not show clustering related to treatment groups. Furthermore, as we have not adjusted for multiple testing these findings may be coincidental, as two statistically significant results (with a significance level $\alpha = 0.05$) out of 39 analyses is what we would expect to occur by chance.

As cytokine signalling is complex and interacts in tightly regulated networks to mediate and regulate cellular processes we assessed the change in correlation patterns over time in both treatment groups. In the placebo group changes in CXCL9, CXCL10 (IP-10) and CXCL11 correlated with changes in TNF, whereas for the Amoxicillin group CXCL9, CXCL10 (IP-10) and CXCL11 formed a network with IL-10. CXCL9, CXCL10 (IP-10) and CXCL11 are Th1 cell-attracting chemokines that may be expressed by macrophages influenced by other pro-inflammatory cytokines like IFN-γ and TNF[9,10]. TNF is a pleiotropic pro-inflammatory cytokine, while IL-10 is known for its anti-inflammatory properties. To our knowledge there are no known direct anti-inflammatory effects of Amoxicillin. Thus our results may suggest that long-term treatment with Amoxicillin may have longstanding effects involving inflammatory mediators, possibly via indirect effects (e.g., gut microbiota in systemic inflammation) that need to be further explored.

In our previous study there was a correlation between IL-6, CXCL13 and CCL27 and LBP-intensity at baseline[5]. The present study showed that decreasing levels of IL-6, CXCL13, CCL27, IL-8 and CCL19 were associated with less pain at 1 year. This is of potential interest to explore since IL-6, CXCL13 and IL-8 have been linked to central sensitization and pain generation, neuropathic pain and hyperalgesia, respectively[11–13]. However, in the adjusted model IL-6 was no longer associated with change in LBP, and the association may have been influenced by association at baseline or regression towards mean of cytokine concentrations.

As the changes in serum cytokine levels overall are small, the alterations we find might not be sufficient to influence pain perception. Also there might be a non-linear relationship between cytokine levels and pain or certain thresholds to influence pain perception[14]. Consequently other cytokines may be linked to pain generation. Furthermore, we measure cytokines systemically, and serum cytokines may not mirror local inflammatory mediators that influence pain generation and perception[15].

Strengths of this study are the longitudinal design with intra-individual comparisons of cytokine levels reducing the likelihood of potential confounding effects of inter-individual differences in lifestyle and environmental exposures. Another asset is that all analyses were done using well-established, validated measurement assays giving robust and reproducible results. We also used a broad panel of cytokines, and have a well characterized patient group.

Limitations include a lack of healthy controls or a control group with cLBP without MCs. Hence, the specificity of our results for MCs is uncertain. Furthermore, a small sample size and not adjusting for multiple testing increase the risk of type 1 errors.

The current study is in line with the results from the AIM-study that infection is not a major cause of cLBP and MCs[7]. Being an exploratory study, these results must be interpreted with caution. However, our findings may contribute to a better understanding of underlying pathomechanisms, and highlight effects on long-term use of antibiotics in humans.

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Declaration of interest

Dr. Løvik Goll has received honoraria from AbbVie, Boehringer Ingelheim, Celltrion, Novartis, Lilly, Orion Pharma, Pfizer and Sandoz outside the submitted work. Dr. Selmer has received honoraria from Roche outside the submitted work. The other authors declare that they have no conflict of interest.

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.joca.2023.01.001.

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